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(71) Applicant: ENZO BIOCHEM, INC.  
325 Hudson Street  
New York, N.Y. 10013(US)

(72) Inventor: Rabbani, Elazar  
69 Fifth Avenue  
New York, N.Y. 10003(US)

(74) Representative: Vossius Vossius Tauchner Heunemann  
Rauh  
Siebertstrasse 4 P.O. Box 86 07 67  
D-8000 München 86(DE)

(54) Heterologous system for the detection of chemically-labeled DNA and other biological materials providing a receptor or target moiety thereon.

(57) The detection and/or identification of materials of interest, such as biological materials, e.g. chemically labeled DNA, which provide a receptor thereon, is improved by employing a heterologous detection system by employing more than one binding reactions, such as reactions involving the affinity of lectin to a glycoprotein or sugar group and the affinity between biotin and avidin or streptavidin, avidin itself being a glycoprotein. Particularly useful in the heterologous detection system are biotinylated lectins, such as biotinylated Concanavalin A, biotinylated dextran, lectin-glycoprotein complexes, such as lectin-avidin and lectin-enzyme complexes and streptavidin-biotinylated enzyme complexes. By way of example, the detection of single-stranded glycosylated DNA hybridized to complementary single-stranded DNA is detected by bringing the hybridized double-stranded DNA into contact with a lectin, such as Concanavalin A, for attachment to the glycosyl group of the glycosylated DNA, followed by contact with a biotinylated dextran, a polysaccharide, and then by contact with a lectin-enzyme complex, such as Concanavalin A-horseradish peroxidase or acid phosphatase. Also, there would be employed an enzyme complex comprising streptavidin-biotinylated horseradish peroxidase. The lectin-enzyme complex would attach itself to the sugar or saccharide of the biotinylated dextran which is also attached to the lectin which is fixed to the glycosyl

groups of the hybridized DNA. The streptavidin-biotinylated enzyme complex would attach itself to the biotin moieties of the biotinylated dextran which, as indicated, is linked to the lectin attached to the glycosyl groups of the target double-stranded DNA.

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Case: 14

Enzo Biochem, Inc.  
New York, U.S.A.

5                    HETEROLOGOUS SYSTEM FOR THE DETECTION OF  
                    CHEMICALLY-LABELED DNA AND OTHER BIOLOGICAL  
                    MATERIALS PROVIDING A RECEPTOR OR TARGET  
                    MOIETY THEREON

BACKGROUND OF THE INVENTION

10                    The detection of labeled molecules, such as  
                    chemically-labeled DNA or other materials or molecules,  
                    containing a receptor or target moiety thereon, either  
                    naturally-occurring or placed thereon, is an important  
                    activity from a commercial, diagnostic and scientific  
                    point of view. For example, biotinylated nucleotides  
                    capable of being incorporated into double-stranded DNA  
                    have been prepared. Single-stranded DNA containing  
15                    such biotinylated nucleotides have been employed,  
                    after hybridization with complementary single-stranded  
                    DNA, to identify various types of DNA material. The  
                    presence of the biotinylated nucleotide in the hybrid-  
                    ized double-stranded DNA is generally detected by a  
20                    reaction involving the affinity between biotin and  
                    avidin, the avidin normally attached to a biotinylated  
                    enzyme, such as biotinylated horseradish peroxidase.  
                    Streptavidin may be substituted for avidin in such a  
                    system. The utilization of streptavidin or avidin  
25                    as a probe for the detection of biotinylated nucleotides  
                    or biotinylated DNA is disclosed in co-owned European  
                    Patent Application Publication No. 0 063 879 published  
                    March 11, 1983, and derived from U.S. application  
                    Serial No. 255,223 filed April 17, 1981. The disclosures

of the above-referred European patent application publication and U.S. application are herein incorporated and made part of this disclosure.

It has also been proposed to label nucleotides and DNA with glycosyl groups or sugar moieties, such as maltose, lactose, mannose, triose, and to detect the thus-labeled nucleotides or DNA with a lectin, such as Concanavalin A, which possesses a strong affinity for sugar groups and glycoproteins. The labeling of DNA with glycosyl groups and detection of the thus-labeled DNA is disclosed in copending, coassigned U.S. patent application Serial No. 391,440 filed June 23, 1982. The disclosures of this U.S. patent application are herein incorporated and made part of this disclosure.

Other techniques for detection of biological materials have also been proposed. For example, European Patent Application Publication No. 0 071 976 published February 16, 1983 involves the covalent binding of biotin to immunologically active material and covalent binding of avidin to an enzyme, such as horseradish peroxidase. Another detection technique is disclosed in European Patent Application Publication No. 0 074 520 published March 23, 1983. This European patent application publication discloses a technique for the detection of human chorionic gonadotropin (HCG) involving a lectin bound to a solid support brought into contact with a sample, such as a urine specimen, suspected of containing HCG. After removal from the support, the resulting lectin-fixed HCG is then brought into contact with an antibody and a color carrier material. The presence of lectin-fixed HCG is indicated by color formation. The disclosures of the above-identified European patent application publications are herein incorporated and made part of this disclosure.

It is an object of this invention to provide improved techniques and materials useful in the identification and/or determination of labeled materials, particularly chemically-labeled biological materials which provide a receptor or target moiety thereon (e.g., chemically-labeled DNA).

It is another object of this invention to provide a rapid diagnostic technique useful for the identification of labeled DNA material, such as biotinylated or glycosylated DNA material.

Still another object of this invention is to provide a kit particularly useful for the identification and/or determination of biotinylated and/or glycosylated DNA.

Yet another object of this invention is to provide a technique of improved sensitivity and versatility for the detection of special chemically-labeled, natural or synthetic, biological materials.

How these and other objects of this invention are achieved will become apparent in the light of the accompanying disclosure.

#### SUMMARY OF THE INVENTION

In accordance with the practices of this invention, there is provided a heterologous detection system and components useful in conjunction therewith and kits for carrying out the heterologous detection system. The heterologous detection system employs the affinity between avidin or streptavidin and biotin, along with the affinity between a lectin and a glycoprotein and/or a glycosyl or sugar group. Particularly useful components in the practices of this invention are the biotinylated lectins and biotinylated sugars (particularly biotinylated polysaccharides, such as biotinylated dextran), biotinylated glycoproteins, and biotinylated enzymes.

Avidin is a glycoprotein having a molecular weight of about 68,000 and a very high affinity for biotin, greatly exceeding that of an antibody for most antigens. Specifically, an avidin molecule provides four binding sites for a biotin molecule. Avidin-biotin affinity is essentially irreversible and comparable to a covalent bond. Proteins, glycoproteins, and enzymes can be conjugated with several molecules of biotin. The special affinity between avidin and biotin provides for the possibility of forming macromolecular complexes between avidin and avidin-containing materials and biotin-containing materials, such as biotinylated enzymes, lectins and polysaccharides. Streptavidin, a molecule closely related to avidin, is preferably substituted for avidin in such complexes, particularly in connection with the detection of biotin-labeled DNA or glycosylated DNA.

Lectins and biotinylated lectins are an important component of the heterologous detection system in accordance with the practices of this invention. Lectins are proteins or glycoproteins with two or more binding sites that recognize a specific sequence of sugar residues. Although originally isolated from plants, lectins have been found in all types of organisms. Lectins which are usefully employed in the practices of this invention include dolichos biflorus agglutinin, which has specificity for the group N-acetylgalactosaminy, lentil lectin which has an affinity for alpha-D-mannose and alpha-D-glucose, as does garden pea lentil lectin. Many other lectins are known and are commercially available. Several commercially available lectins and the specific sugar residues they recognize are set forth in accompanying Table I:

TABLE I

	<u>Lectin</u>	<u>Sugar Specificity</u>
	Concanavalin A (from Jack beans)	alpha-D-glucose and
5	Soybean Lectin	alpha-D-mannose D-galactose and N-acetyl D-galactosamine
	Wheat Germ Lectin	N-acetylglucosamine
	Lotus Seed Lectin	fucose
10	Potato lectin	

Also of interest in the practices of this invention are glycolipids which are oligosaccharide-containing molecules found on the surface of all plasma membranes. More specifically, glycolipids are present in the outer half of the bilayer of the cell membrane with the sugar group exposed on the cell surface. The neutral glycolipids with polar head groups consisting of from 1-15 or more neutral sugars are found widely distributed in the plasma membranes of both eucaryotic and procaryotic cells. Some glycolipids are found only in certain mammals and usually in only certain tissues thereof. For example, galactocerebroside, one of the simplest glycolipids containing only galactose and its polar head group, is the main glycolipid in myelin. More complex glycolipids are the gangliosides of which about 30 have been identified. Lectins would be useful for attachment to the exposed glycolipid oligosaccharide groups as carriers or substrates for cell membrane identification because of the normal affinity between lectins and sugar groups.

Of special interest in the practices of this invention are the glycoproteins, particularly the enzymes, such as glucose oxidase, peroxidase, horseradish peroxidase, alkaline phosphatase, acid  
5 phosphatase and  $\beta$ -galactosidase. The above-mentioned enzymes are merely exemplary of the many known glycoproteins or enzymes, which are useful in the practices of this invention.

One aspect of the practices of this invention involves biotinylated enzymes. Various techniques may be employed in the preparation of biotinylated enzymes. An exemplary preferred technique is disclosed in co-pending, co-assigned U.S. patent application Serial No. 486,924 filed April 20, 1983,  
15 the disclosures of which are herein incorporated and made part of this disclosure. This application discloses a technique for the biotinylation of enzymes wherein the enzyme is fixed to a solid substrate, preferably through a competitive inhibitor therefor,  
20 which protects the active site of the enzyme during the exposure of the enzyme to biotin. After the enzyme has been biotinylated, it is released from attachment to the competitive inhibitor which remains fixed to the solid substrate. Alternatively, while  
25 the biotinylated enzyme is still fixed to the solid substrate, further reaction or complex formation such as the addition of avidin or streptavidin to the fixed biotinylated enzyme, can be performed. After the attachment of avidin or streptavidin to  
30 the fixed biotinylated enzyme, the resulting complex can be released from the solid substrate. By employing this technique, various complexes can be prepared. For example, the fixed enzyme can be bound to a lectin rather than to biotin with the resulting formation  
35 of an enzyme-lectin complex which can then be removed from attachment to the solid substrate and employed as the reagent in the heterologous system in accord-

ance with this invention. This technique is also applicable for the preparation of biotinylated lectins and biotinylated polysaccharides, among others. These products, as well as the enzyme  
5 lectin complex, can be employed to produce other larger complexes including other enzymes, glycoproteins and the like.

Techniques for the employment of an enzyme complex in the identification of a labeled DNA are  
10 disclosed in co-pending, co-assigned U.S. patent application Ser. No. 490,712 filed May 2, 1983. The disclosures of this patent application are herein incorporated and made part of this disclosure. This patent application describes techniques for the  
15 analysis of genetic material, such as DNA and RNA. The genetic material to be analyzed or identified is denatured, fixed to a substrate and hybridized with a probe, such as a chemically-labeled probe having a nucleotide sequence complementary to the genetic  
20 material to be identified. After the hybridization between the chemically-labeled probe and the DNA to be identified, an enzyme component effective on contact with a chromagen to produce an insoluble color precipitate or color product is attached to the probe.  
25 These techniques are applicable to the heterologous detection system in accordance with this invention and provide for the analysis and detection of genetic material with improved sensitivity, definition, accuracy and/or speed. The heterologous detection  
30 system of this invention is especially useful in such well known analytical DNA techniques, such as Southern blot analysis, Northern blot analysis, Western blot analysis, colony hybridization, plaque lifts, cytoplasmic dot hybridization and other ana-  
35 lytical techniques for for the identification of genetic material such as DNA and RNA.



The following examples indicate the practices of this invention and are directed to the detection of various materials, such as human chorionic gonadotropin and DNA. In the tests employing biotinylated DNA and glycosylated DNA, the labelled DNA is hybridized to its complementary DNA utilizing a blot test technique in which the hybridized DNA is fixed to a suitable substrate. Example I involves a combination consistent with the broad heterologous detection system of this invention including the use of antibodies to a specific biological material in fixing the material to a substrate, the use of a lectin capable of attachment to the biological material and the use of a signal moiety specific for the lectin.

Example No. 1

Monoclonal antibodies to human chorionic gonadotropin (HCG) were bound to dextran (Sephacrose) beads employing cyanogen bromide. In addition to its use as a coupling agent, the cyanogen bromide destroys most of the affinity of the Sepharose for the lectin Concanavalin A. Glass beads or polyacrylamide beads could be used in place of dextran beads to minimize control levels of Concanavalin A binding.

250  $\mu$ l of the Sepharose beads were incubated with 20  $\mu$ l (~670 units) of a preparation containing HCG for about 45 minutes at room temperature. In the control test no hormone was added or present. The Sepharose beads were washed five times with 2.5 ml aliquots of phosphate buffered saline (PBS). After each washing the beads were separated by centrifugation at ~ 1000 xg for one minute. The supernatant liquids were discarded and the beads were then washed once with 2 ml of 0.2 M imidazole buffer, pH 6.8, 1 mM Mn<sup>++</sup>, 1 mM Ca<sup>++</sup>. The beads were again separated by centrifugation. The

imidazole Mn-Ca buffer was selected to maximize Concanavalin A binding.

5 The beads were then treated with 50 g of iodinated Concanavalin A, specific activity of 6400 cpm/ug in 400  $\mu$ l of 0.2 M imidazole buffer, ph 68., 1 mM Mn++ and 1 mM Ca++. Following incubation for about 30 minutes at room temperature, the samples were washed three times with 5 ml aliquots of 0.2 M NaCl. The radioactivity in the supernatant from the last wash was approximately 67 cpm/100  $\mu$ l. The beads were then separated by centrifugation, suspended in 0.2 M NaCl and counted. For the sample containing HCG, 231,840 cpm were bound and for the control, 117,150 cpm were bound.

15 In addition to the radioactive determination of the bound Concanavalin A, the presence of Concanavalin A bound to the HCG affixed to the beads could be determined in accordance with the practices of this invention by adding and fixing an enzyme to the bound Concanavalin A. The presence of the thus-bound enzyme could be determined or evidenced by conventional means, i.e., by providing a suitable enzyme chromogen-containing substrate for reaction with the Concanavalin A bound enzyme. In the above example a heterologous detection system is employed involving antibodies for linking with the HCG, the HCG linked to the lectin, Concanavalin A, and the lectin linked to a glycoprotein, the enzyme.

30 In the above example the determination of the amount of bound Concanavalin A would detection with streptavidin-biotinylated horseradish peroxidase enzyme complex. In accordance with this aspect of the heterologous detection system of this invention, the streptavidin attaches itself to the Concanavalin A along with the biotinylated horseradish peroxidase and the thus-fixed streptavidin biotinylated horse-radish peroxidase is evidenced by a suitable chromogen

or chromogenic or color yielding reaction involving the fixed horseradish peroxidase.

Example No. 2

5 A complex formed by biotinylated DNA and streptavidin-biotin horseradish peroxidase allows the detection of 100 pcg of DNA bound to nitrocellulose paper. This sensitivity of biotinylated DNA with streptavidin-biotin horseradish peroxidase is indicated in the above-identified U.S. patent application Serial No. 490,712. Glycosylated DNA and lectin for the determination of the glycosylated DNA is not as sensitive. The use of the lectin system for the detection of glycosylated DNA is advantageous, however, since the enzyme, being also glycosylated, 15 can be directly bound to the glycosylated DNA-lectin without further manipulation. The special component of this invention, biotinylated lectin, offers a special technique in accordance with the practices of this invention to improve the glycosylated DNA-lectin system for the detection of DNA. 20

DNA dot blots were prepared using naturally glycosylated DNA ( $T_4$  Phage DNA) and nick translated biotinylated DNA. The nitrocellulose or similar filters containing the DNA dot blots were blocked 25 to prevent non-specific binding of the lectin, Concanavalin A, thereto. This procedure involves blocking at about 50°C. with 2% BSA, washing once with SSC and contacting with 0.1% of the surfactant Triton X-100. The DNA dot blots were then processed.

30 The DNA dot blots were contacted with complementary DNA which had been biotinylated to about 20% biotinylation by nick translation. These tests are indicated in Table II, below.

TABLE II

	<u>Biotinylated DNA</u>	<u>Carrier DNA</u>
	2 ng	4.5 ng
	1 ng	4.5 ng
5	500 pcg	4.5 ng
	250 pcg	4.5 ng
	125 pcg	4.5 ng
	62.5 pcg	4.5 ng
	31.25 pcg	4.5 ng
10	15.625 pcg	4.5 ng Control

After blocking, the streptavidin-biotin horseradish peroxidase complex was added in the amount 20 ul/cm<sup>2</sup> to the biotinylated DNA blots and incubated at 37°C. for 45 minutes. The blots were rinsed three times at 5 minutes each in a high salt buffer and two times at 5 minutes each in a low salt buffer at room temperature. Thereupon, DAB (0.5 mg/ml in 10 mM Tris at a pH 7.6 plus 0.02% CoCl<sub>2</sub>) was applied for 10 minutes at about 0°C. in the dark. Afterwards, 0.02% H<sub>2</sub>O<sub>2</sub> was added for immediate detection of the complex.

The glycosylated DNA blots employing the glycosylated T<sub>4</sub> phage DNA were tested as indicated in the following Table III.

TABLE III

	<u>T<sub>4</sub> phage DNA</u>	<u>Carrier DNA</u>
	500 pcg	4.5 ng
	250 "	4.5 ng
5	125 "	4.5 ng
	62.5 "	4.5 ng
	31.25 "	4.5 ng
	15.625 "	4.5 ng
	7.8125 "	4.5 ng
10	3.9 "	4.5 ng
	0	Control

After blocking of the blots, biotinylated Concanavalin A was applied to the strip of blots in the amount of 20  $\mu\text{l}/\text{cm}^2$  or 100  $\mu\text{g}/\text{ml}$  in TCMN solution made up of 5 mM Tris pH 7.0, 1 mM  $\text{MnCl}_2$ , 1 mM  $\text{CaCl}_2$  and 100 mM NaCl and the blots were incubated at 37°C. for 1 hour in a damp atmosphere. The experimental results indicated that rinsing with TCMN buffer (100-200 ml) resulted in high background (non-specific binding of Concanavalin A to the nitrocellulose paper).

Accordingly, 1 mM glucose solution was substituted for the wash buffer in another set of experiments. In this set of experiments the glycosylated DNA blots on nitrocellulose filter paper were blocked at 50°C. with 2% BSA, washing once with SSC and 0.1% surfactant, Triton X-100. Biotinylated Concanavalin A in the amount 100  $\mu\text{g}/\text{ml}$  or 20  $\mu\text{l}/\text{cm}^2$  in TCMN buffer was applied at 37°C. for 60 minutes, followed by five rinses or soakings of 5 minutes each with 1 mM  $\alpha$ -D-glucose and three soakings or rinses at 5 minutes each with TCMN. The streptavidin-biotin-horseradish peroxidase complex was then applied at 37°C. for 30 minutes and washed with a high salt buffer and a low salt buffer, followed by detection of

the blot employing DAB-H<sub>2</sub>O<sub>2</sub>, as described hereinabove. The observed results indicated a greatly increased sensitivity for the detection of the glycosylated DNA.

5           The above examples illustrate the versatility and broad applicability of the practices of the heterologous detection system of this invention, which does not depend upon only one ligand or reaction or affinity. Where a target material has attached  
10 thereto a biotin moiety, a homologous detection system is known which involves an enzyme complex comprising avidin or streptavidin-biotin enzyme, e.g., streptavidin-biotin horseradish peroxidase. Where  
15 the target material is glycosylated DNA, another homologous detection system is known involving lectin for attachment to the glycosylated DNA or other sugar groups attached to the target material, and an enzyme or enzyme complex capable of attachment to the lectin. The lectin joins both the target moiety, glycosylated  
20 DNA, and the signal moiety comprising the enzyme.

          In the heterologous detection system in accordance with this invention, however, a preferred embodiment thereof resides in the use of the combination involving the affinity between biotin and avidin  
25 or biotin and streptavidin and the affinity between a lectin and a glycoprotein. This heterologous detection system in effect unexpectedly combines the features of two homologous detection systems. For example, when the target contains biotin groups,  
30 such as biotinylated DNA, the detection system would employ a complex, which contains avidin or streptavidin and biotinylated lectin. This complex would fix or attach itself to the biotin portion of the target and would be elicited by bringing into contact  
35 therewith and fixing to the lectin portion of the complex an enzyme containing a glycosyl group, such as horseradish peroxidase. This enzyme would then

be activated or employed to signal its attachment to the avidin biotinylated lectin complex. Additionally, the avidin biotinylated lectin complex could be elicited by contact with the enzyme complex, streptavidin-  
5 biotinylated enzyme, such as streptavidin-biotin horseradish peroxidase.

For the determination of a glycosylated or sugar-containing target material, biotinylated lectins could be attached directly thereto, followed by  
10 attachment of a glycoprotein, such as horseradish peroxidase enzyme, to the thus-fixed lectin. The presence of the attached enzyme is directly elicited by suitable color change reaction. Additionally, the biotinylated lectin bound to the target material  
15 could be brought into contact with an enzyme complex, e.g., streptavidin-biotin horseradish peroxidase. This system would provide two signals, one effected by the attachment of the enzyme directly to the lectin and the other by the attachment of the streptavidin-  
20 biotin enzyme complex to the biotin moiety of the biotinylated lectin. Also, because a lectin has an affinity for glycoproteins and since avidin and streptavidin are glycoproteins, an unlabelled lectin could be employed with or in place of a biotinylated  
25 lectin.

The applicability of the biotinylated lectins and biotinylated enzymes in the practices of heterologous detection system of this invention has been indicated above. Another special component, a biotinylated polysaccharide, such as biotinylated dextran  
30 or biotinylated agarose, also provides advantages in the practices of this invention. For example, in the detection of a sugar-labelled target material, a lectin would be brought into contact with the glycosylated DNA for attachment thereto. Dextran or  
35 biotinylated dextran would then be brought into contact with the lectin, now fixed to the glycosylated DNA. The dextran would attach itself to the lectin

and the thus-attached dextran would be elicited by contact with a lectin-enzyme complex, the lectin of the lectin-enzyme complex attaching itself to the dextran. If a biotinylated dextran were employed in place of or in addition to the dextran, the biotinylated dextran attached to the target-fixed lectin could be elicited by contact with a subsequently added lectin enzyme complex and by contact with an avidin or streptavidin-biotin-enzyme complex. The avidin or streptavidin-biotin complex would attach itself to the biotin moiety of the biotinylated dextran. Because many sites are offered by the dextran or biotinylated dextran for attachment with the probe or signal-generating lectin-enzyme complex and/or avidin or streptavidin-biotin enzyme complex, a substantial signal amplification or enlargement is obtainable.

In accordance with the practices of this invention, therefore, signal enlargement or amplification is obtainable by employing in combination a lectin or biotinylated lectin, a dextran or biotinylated dextran for attachment thereto and for further attachment of a lectin complex, or an avidin or streptavidin enzyme complex or an avidin-biotin dextran complex with eventual utilization of a lectin-enzyme complex or a streptavidin or avidin-biotin enzyme complex.

Many combinations may be employed in the practices of this invention for the detection of biotinylated, glycosylated or sugar-labeled target materials. Such combinations can include lectin, a biotinylated lectin; a dextran or a polysaccharide, a biotinylated dextran or polysaccharide, avidin, a lectin-avidin complex, a lectin-enzyme complex, an avidin or streptavidin-biotinylated-enzyme complex, and a biotinylated glycoprotein. Particularly in association with lectins and biotinylated lectins,



there may also be employed immunobiologically active materials, such as antigens, antibodies and anti-antibodies, in the combination heterologous detection system of this invention. See for example, U.S.

- 5 Patent 4,289,747, the disclosures of which are herein incorporated and made part of this disclosure.

As will be apparent to those skilled in the art in the light of the foregoing disclosure, many modifications, substitutions and alterations  
10 are possible in the practice of this invention without departing from the spirit or scope thereof.

So. 229

WHAT IS CLAIMED IS:

1. A complex comprising a biotinylated lectin.
2. The complex according to claim 1, further comprising one or more members of the group consisting of avidin, streptavidin and a biotinylated enzyme.
3. The complex according to Claim 2, characterized in that said enzyme is selected from the group consisting of acid phosphatase, glucose oxidase, horseradish peroxidase, alkaline phosphatase,  $\beta$ -galactosidase, chloroperoxidase, and monamine oxidase.
4. The complex according to Claims 1 or 2, characterized in that said lectin is selected from the group consisting of Concanavalin A, soybean agglutinin, wheat germ agglutinin, ulex europaeus agglutinin I, peanut agglutinin, ricinus communis agglutinin I, phaseolus vulgaris agglutinin, japonica agglutinin, lotus seed lectin, and potato lectin.
5. A complex comprising a lectin and a glycoprotein selected from the group consisting of avidin and streptavidin.
6. The complex according to claim 5, further comprising one or more members of the group consisting of an enzyme and a biotinylated enzyme.

7. The complex according to Claim 5 or 6, characterized in that said lectin is selected from the group consisting of Concanavalin A, soybean agglutinin, wheat germ agglutinin, ulex europaeus agglutinin I, peanut agglutinin, ricinus communis agglutinin I, phaseolus vulgaris agglutinin, japonica agglutinin, lotus seed lectin, and potato lectin.

8. The complex according to Claim 6, characterized in that said enzyme is selected from the group consisting of acid phosphatase, glucose oxidase, horseradish peroxidase, alkaline phosphatase,  $\beta$ -galactosidase, chloroperoxidase, and monamine oxidase.

9. A complex comprising a lectin and a biotinylated polysaccharide.

10. The complex according to Claim 9, further comprising one or more members of the group consisting of an enzyme, a biotinylated enzyme, avidin and streptavidin.

11. The complex according to claim 9 or 10, characterized in that said lectin is selected from the group consisting of Concanavalin A, soybean agglutinin, wheat germ agglutinin, ulex europaeus agglutinin I, peanut agglutinin, ricinus communis agglutinin I, phaseolus vulgaris agglutinin, japonica agglutinin, lotus seed lectin, and potato lectin.

12. The complex according to Claim 10, characterized in that said enzyme is selected from the group consisting of alkaline phosphatase, acid

phosphatase, glucose oxidase,  $\beta$ -galactosidase, horse-radish peroxidase, monamine oxidase and chloro-peroxidase.

13. A complex comprising a lectin, a saccharide, a biotinylated enzyme, and a glycoprotein selected from the group consisting of avidin and streptavidin.

14. The complex according to Claim 13, characterized in that said enzyme is selected from the group consisting of alkaline phosphatase, acid phosphatase, glucose oxidase,  $\beta$ -galactosidase, horse-radish peroxidase, monamine peroxidase and chloro-peroxidase.

15. The complex according to claim 13, characterized in that said saccharide is selected from the group consisting of a monosaccharide and a polysaccharide.

16. The complex according to claim 13, characterized in that said lectin is selected from the group consisting of Concanavalin A, soybean agglutinin, wheat germ agglutinin, ulex europaeus agglutinin I, peanut agglutinin, ricinus communis agglutinin I, phaseolus vulgaris agglutinin, japonica agglutinin, lotus seed lectin, and potato lectin.

17. A complex comprising a biotinylated dextran.

18. The complex according to claim 17, further comprising a lectin, an enzyme, and a glycoprotein selected from the group consisting of avidin and streptavidin.

19. The complex according to claim 18, characterized in that said enzyme is biotinylated.

20. A complex according to claim 19, characterized in that said lectin is biotinylated.

21. The complex according to claim 19, further comprising a lectin-attached enzyme.

22. A hybridized double-stranded DNA sequence comprising a strand characterized by at least one nucleotide labeled with a saccharide moiety, said nucleotide associated with a complex comprising a biotinylated lectin, a biotinylated enzyme, and a glycoprotein selected from the group comprising avidin and streptavidin.

23. The DNA sequence according to claim 22, characterized in that said complex further comprises a biotinylated dextran.

24. The DNA sequence according to Claim 22 or 23, characterized in that said saccharide moiety is selected from the group consisting of a mono-saccharide and a polysaccharide.

25. The DNA sequence according to Claim 22 or 23, characterized in that said enzyme is selected from the group consisting of alkaline phosphatase, acid phosphatase, glucose oxidase,  $\beta$ -galactosidase, horseradish peroxidase, monamine oxidase and chloroperoxidase.

26. A hybridized double-stranded DNA sequence comprising a strand characterized by at least one biotinylated nucleotide having attached

thereto a complex comprising a biotinylated lectin, an enzyme, and a glycoprotein selected from the group consisting of avidin and streptavidin.

27. A hybridized double-stranded DNA sequence comprising a strand characterized by at least one nucleotide labeled with a saccharide moiety, and, having attached to said labeled nucleotide, a complex comprising lectin, dextran and an enzyme, a portion of the lectin being attached to said saccharide and said dextran, and another portion of the lectin being attached to said dextran and said enzyme.

28. The DNA sequence according to Claim 27, characterized in that said saccharide moiety is selected from the group consisting of monosaccharide and polysaccharide.

29. A method of determining the presence of a biotinylated nucleotide comprising: contacting said biotinylated nucleotide with a complex comprising a biotinylated lectin, an enzyme, and a glycoprotein selected from the group consisting of avidin and streptavidin.

30. A method of determining the presence of a glycosylated or saccharide labeled nucleotide comprising: contacting the glycosyl or saccharide moiety of said nucleotide with a complex comprising lectin and an enzyme.

31. The method according to Claim 30, characterized in that said lectin is biotinylated, said enzyme is biotinylated and said complex additionally comprises a glycoprotein selected from the group comprising avidin or streptavidin.

(19)



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(71) Applicant: **ENZO BIOCHEM, INC., 325 Hudson Street,**  
**New York, N.Y. 10013 (US)**

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(72) Inventor: **Rabbani, Elazar, 69 Fifth Avenue, New York,**  
**N.Y. 10003 (US)**

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(74) Representative: **Vossius & Partner,**  
**Siebertstrasse 4 P.O. Box 86 07 67,**  
**D-8000 München 86 (DE)**

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(54) **Heterologous system for the detection of chemically-labeled DNA and other biological materials providing a receptor or target moiety thereon.**

(57) The detection and/or identification of materials of interest, such as biological materials, e.g. chemically labeled DNA, which provide a receptor thereon, is improved by employing a heterologous detection system by employing more than one binding reactions, such as reactions involving the affinity of lectin to a glycoprotein or sugar group and the affinity between biotin and avidin or streptavidin, avidin itself being a glycoprotein. Particularly useful in the heterologous detection system are biotinylated lectins, such as biotinylated Concanavalin A, biotinylated dextran, lectin-glycoprotein complexes, such as lectin-avidin and lectin-enzyme complexes and streptavidin-biotinylated enzyme complexes. By way of example, the detection of single-stranded glycosylated DNA hybridized to complementary single-stranded DNA is detected by bringing the hybridized double-stranded DNA into contact with a lectin, such as Concanavalin A, for attachment to the glycosyl group of the glycosylated DNA, followed by contact with a biotinylated dextran, a polysaccharide, and then by contact with a lectin-enzyme complex, such as Concanavalin A-horseradish peroxidase or acid phosphatase. Also, there would be employed an enzyme complex comprising streptavidin-biotinylated horseradish peroxidase. The lectin-enzyme complex would attach itself to the sugar or saccharide of the biotinylated dextran which is also attached to the lectin which is

fixed to the glycosyl groups of the hybridized DNA. The streptavidin-biotinylated enzyme complex would attach itself to the biotin moieties of the biotinylated dextran which, as indicated, is linked to the lectin attached to the glycosyl groups of the target double-stranded DNA.

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European Patent  
Office

# EUROPEAN SEARCH REPORT

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Application Number

EP 85 10 1353

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 4)
P, X Y	EP-A-0 123 300 (ENZO BIOCHEM INC.) * Whole document, especially claims 1-9 * ---	1-31	G 01 N 33/535 C 12 Q 1/68 G 01 N 33/53
Y	EP-A-0 122 614 (ENZO BIOCHEM INC.) * Whole document * ---	1-31	
Y	WO-A-8 404 970 (YALE UNIVERSITY) * Page 5, line 7 - page 6, line 29 * ---	1-31	
Y	EP-A-0 063 879 (YALE UNIVERSITY) * Whole document, especially page 30, lines 12-35 * ---	1-31	
P, Y	EP-A-0 128 332 (ENZO BIOCHEM INC.) * Page 19, line 23 - page 21 * ---	1-31	
E	WO-A-8 503 356 (MOLECULAR BIOSYSTEMS, INC.) * Whole document * -----	1-31	
The present search report has been drawn up for all claims			TECHNICAL FIELDS SEARCHED (Int. Cl. 4)
			C 12 Q G 01 N
Place of search THE HAGUE		Date of completion of the search 28-07-1988	Examiner OSBORNE H.H.
CATEGORY OF CITED DOCUMENTS			
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			